# Tobacco Budworm: Nocturnal Behavior of Laboratory-Reared Irradiated and Native Adults in the Field



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## ABSTRACT

Nocturnal behavior of native (N) and laboratory-reared, irradiated ($CO_{60}$), and released (IR) adult tobacco budworms, *Heliothis virescens* (F.), was studied in small diverse cropping systems near Isabela, P.R., and at St. Croix, U.S. Virgin Islands. When several techniques were used to study the diel patterns of feeding, oviposition, copulation, flying, and sitting, asynchrony in some cases was apparent between strains and between sexes within strains. This asynchrony contributed to the highly significant difference in mating competitiveness between the N and IR insects, but population age structure (based on prior mating status of females), concentrations of females, dark-adaptation of adults, and attractiveness of host plants greatly influenced the dispersal and competitiveness. Establishment of these behavioral criteria for native adults of various species will prove useful for determining numerous responses in the field, such as pheromone response and mating competitiveness, and should result in the development of competitive laboratory strains.

KEYWORDS: Tobacco budworm, *Heliothis virescens*, nocturnal insect behavior, mating interaction of native and laboratory-reared tobacco budworms.

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# TOBACCO BUDWORM: NOCTURNAL BEHAVIOR OF LABORATORY-REARED IRRADIATED AND NATIVE ADULTS IN THE FIELD

By P. D. Lingren, J. R. Raulston, A. N. Sparks, and F. I. Proshold[1]

## INTRODUCTION

The lack of adequate methods for studying nocturnal behavior of most of our adult nocturnal insects has contributed to a limited knowledge of their behavior in the field. Now, however, we have techniques for rearing (*5*),[2] marking (*1*), studying sperm transfer (*4*), and observing nocturnal behavior (*3*, *6*, *10*) that can provide us with more information. In fact, some aspects of nocturnal behavior, such as diel activity patterns of feeding, oviposition, precopulatory activity, and mating, have been reported for a caged (primarily laboratory reared) population of tobacco budworms, *Heliothis virescens* (F.) (*2*). Also, Raulston et al. (*6*) demonstrated an asynchrony in the time of mating of native and laboratory-reared tobacco budworms and showed that the mating behavior of females is greatly influenced by prior mating and sperm content. The sum of these studies has established certain criteria for comparing the interactions and competitiveness of irradiated laboratory-reared and released tobacco budworms with that of their native counterparts. We now report the results of studies of the nocturnal behavior of native (N) and laboratory-reared, irradiated ($CO_{60}$), released (IR) adult tobacco budworms in small diverse cropping systems.

## METHODS AND MATERIALS

### Study Area

Tests were conducted at three locations in support of a pilot test using sterile releases of tobacco budworm adults on St. Croix, U.S. V.I. The tests were conducted to determine possible differences in the nocturnal behavior of N and IR that might influence mating interaction between the two strains.

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[1]All the authors are research entomologists, located as follows: Western Cotton Research Laboratory, 4135 E. Broadway Road, Phoenix, Ariz. 85040; Cotton Insects Research Laboratory, P.O. Box 1033, Brownsville, Tex. 78520; Southern Grain Insects Research Laboratory, Georgia Coastal Plain Experiment Station, Tifton, Ga. 31794; and Federal Experiment Station, P.O. Box H, Kingshill, St. Croix, U.S. V.I. 00850, respectively.

[2]Italic numbers in parentheses refer to Literature Cited, p. 16.

### Test Site No. 1

Studies were conducted the nights of May 13 to 16, 1974, in a 1.6- by 1.1-km area on the agricultural experiment substation of the College of Agricultural Sciences, University of Puerto Rico, Isabela, and on the adjoining U.S. Department of Agriculture (USDA) location (fig. 1). The site contained a diverse cropping system, small plots (0.2 to 1 ha) with a variety of crops, among which pigeon pea, *Cajanus cajan* (L.) Millsp., tobacco, and tomato are primary host plants of the tobacco budworm.

### Test Site No. 2

Another test was made the night of June 21 about 11.3 km east of the main study area in three tobacco patches of about 0.2 ha each to obtain a direct comparison of N male response to laboratory-reared females held under light and dark conditions before being placed on mating tables.

### Test Site No. 3

In addition, studies were made for 25 nights between Apr. 11 and May 28, 1974, on the nearby 220-$km^2$ island of St. Croix, U.S. V.I., in about 0.1-ha of pigeon pea and a wild host plant, *Bastardia* sp., that were spread over a 25.9-$ha^2$ area.

## Tobacco Budworm Rearing, Handling, and Releases

The laboratory-reared insects used in the study originated from a culture, collected near Tucson, Ariz., that had been reared at Tucson and Brownsville (*5*), sexed, packaged as pupae, and shipped to St. Croix (*7*) where they were allowed to emerge in an open-air insectary. Irradiation was done early in the morning within 24 h of first emergence with about 25 krad of $CO_{60}$. All released insects were marked internally with Calco Oil Red® dye (*1*), and all releases were made near sundown. All adults that were to be released were provided a 10-percent sugar-water solution as food during the interval immediately after irradiation and before release.

When tests were made at St. Croix, 10,000 to 20,000 1-day-old moths of each sex (light adapted by exposure to natural photoperiods after emergence until release) were transported immediately after irradiation to 25 to 50 sites, placed in release cages of the type described by Skov et al. (*9*), and released.[3]

When tests were made in May in Puerto Rico, adults were packaged in 21- by 25- by 40-cm cardboard boxes that had six 2-cm portholes and were airshipped to Puerto Rico from St. Croix. There they were transported to the test via an air-conditioned vehicle and held at local ambient conditions for from several hours to 2 days before the releases. The first release at Isabela (May 14) consisted of 6,000 2-day-old moths of each sex that had been dark-adapted (held in a dark room for about 4 h) before release. The female were released in

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[3]Numbers of moths released varied, depending on the supply from the rearing facility; numbers of release sites were varied to obtain optimum distribution of the numbers available.

Pigeon Pea No. I (about 0.2 ha, fig. 1) by personnel walking between rows and dumping the moths onto the plants at 7 p.m.; the males were released at 7:15 p.m. at a crop-free site 110 m east of the female release site by allowing them to disperse freely from the holding cartons. Their flight was observed visually; one observer was stationed at the female release site. The second release in this area (May 16) consisted of about 2,000 1-day-old moths of each sex. The females were dark-adapted as before, but the males were merely held outside in a shady area in cartons fitted with a screen-covered top. After release, flight was not observed. Thus, by manipulating releases and determining the time and type of matings that occurred throughout the night, all possible combinations of natural photoperiod-adapted (L) and dark-adapted (D) laboratory (IR) adults of both sexes and N adults were tested except (L-IR) females x (D-IR) males.

## Mating Tables

One of the new techniques used to study the nocturnal behavior of the released IR and N tobacco budworms was mating tables (10). On May 13, before the first release at Isabela, seven such tables were placed at various locations (fig. 1). Each table was baited nightly from May 14 to 16 at about 7:30 p.m. with 50 2-day-old virgin L-IR females with one set of wings removed clipped-wing). The tables were placed away from the male release site as follows: (1) in Pigeon Pea No. II (261 m S-SW); (2) in papaya (117 m S.); (3) at edge of tomato (402 m SW.); (4) in plowed field (484 m N-NW.); (5) in tobacco (512 m S-SW.); (6) in Pigeon Pea No. III (648 m SW.); and (7) in pasture (784 m NE.). Tables were examined hourly from 8 p.m. until 4 a.m. for for copulating pairs, and the male type (N or IR), time, and location were recorded. Data were then summarized hourly throughout the test period.

Also on June 21, two mating tables were placed 6.1 m apart in each of three separate tobacco plots. One table at each plot was baited with 1-day-old virgin IR clipped-wing females that had been dark-adapted for 3 h before they were placed on the tables at about 7:45 p.m.; the other table was baited with females that had been held under natural light. The tables were examined hourly from 8 p.m. until 4 a.m., and the numbers of females calling and mating were recorded.

## Action Sites

A second new technique used to study the behavior of the released and N insects was "action sites." On May 13, just before the first release at Isabela, all major concentrations of host plants (plots) in the test area were observed for state of maturity of plants and natural infestations of immature tobacco budworms. Four action sites were then selected where the plants were blooming or fruiting and were infested with immature tobacco budworms. These sites were located at various distances from the male release site as follows (fig. 1): Pigeon Pea No. I, the female release site (110 m W.); Pigeon Pea No. II (261 m S.); tomato (420 m SW.); and tobacco (512 m S-SW.). Numerous other plots that contained host plants were found, but the state of maturity and lack of natural infestation of budworm larvae suggested that only these four sites, plus Pigeon Pea No. III, were attractive to adult tobacco budworms.

On May 13, an observer was stationed at each of the action sites. This observer searched the site for about 45 min of each hour from 7:15 p.m. until

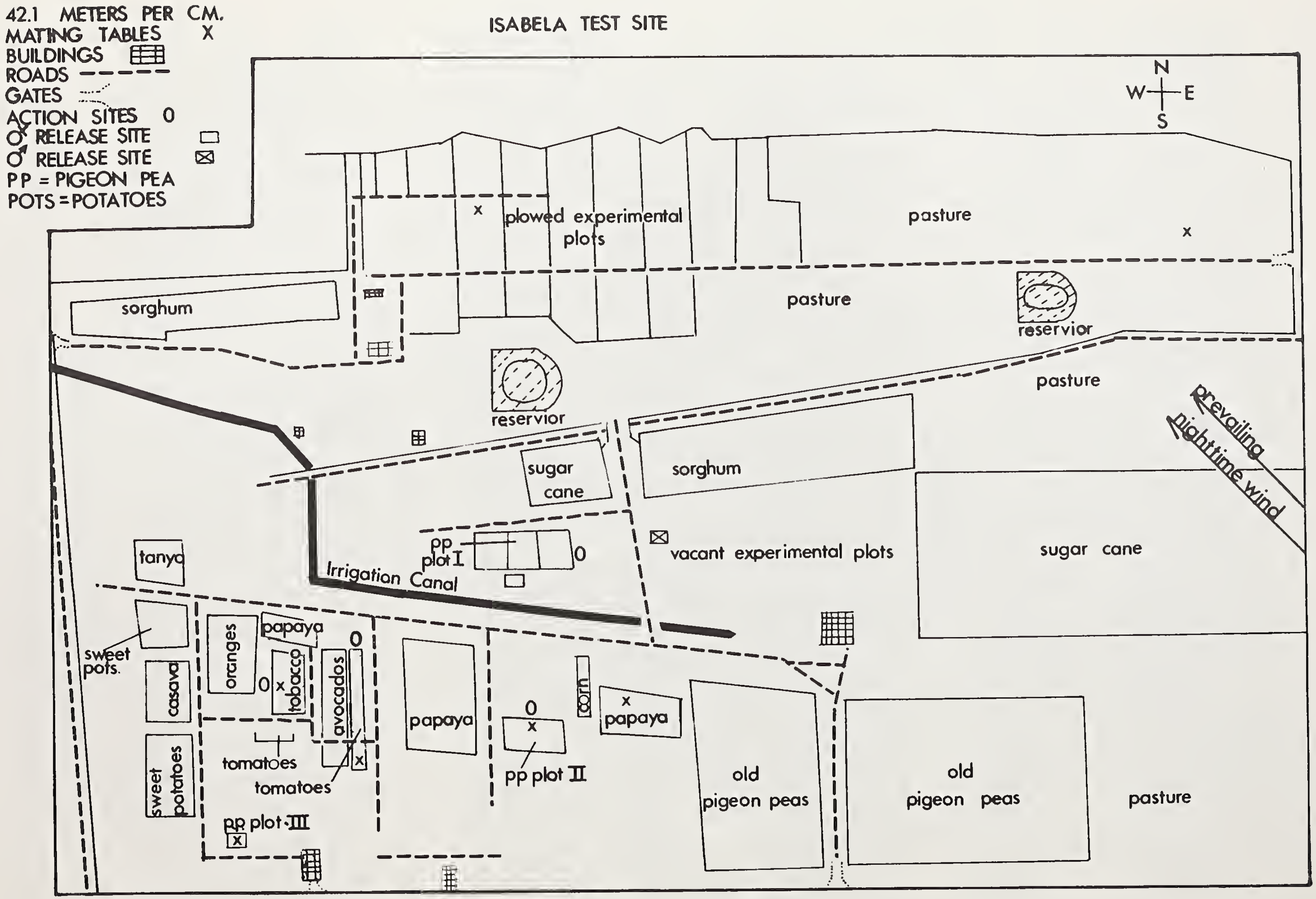


Figure 1.--Experimental test site at Isabela, P.R., showing cropping system, action sites, females release site, male release site, and location of mating tables.

8 a.m. Atlantic standard time (A.s.t.) by using a 6-v headlamp fitted with a 6.4-cm polished reflector as an aid in identifying adult activity. He observed and recorded the following activities: Feeding, oviposition, mating, flying, and sitting. Moths were captured and placed in 14.8-ml plastic cups so sex, location, time of capture, and type of activity could be recorded later in the laboratory; in addition, captured females were dissected so we could determine their prior mating status (number of spermatophores). Several of the plots of host plants that had appeared unattractive were observed for adult activity at various intervals during the night. These same procedures were continued each night through May 17 except that all moths after the first release were identified as laboratory-reared or native and observations were begun at about 6.15 p.m. Similar action sites were set up and manned on St. Croix, but the observations were made between 6:15 p.m. and 8 a.m. in four plots of pigeon pea and one plot of *Bastardia* sp.; the same data were recorded.

### Analysis of Data

Data from all tests were analyzed when appropriate by the G-test for frequency distribution (*11*). Data on L adults from the Isabela and St. Croix tests were summed to establish diel patterns of activity for the behavioral traits observed. These data were reported as the percentage of the total activity of each specific type that was observed each hour of the night for both N and IR males and females. Data from the mating tables were used in conjuction with head counts (adult collections at action sites) and records of mating activity at the action sites to establish patterns of dispersal and mating response for N and IR adults. Data from the June 21 test were summarized to determine the effects of dark adaptation on time and intensity of calling and the response of N males to the calling.

## RESULTS AND DISCUSSION

### Diel Patterns of N and IR Adults

#### Feeding and Oviposition

N adults began feeding between 7 and 8 p.m., but peak feeding activity of females occurred about 1 h later (8 to 9 p.m.) than that of males (7 to 8 p.m.) (fig. 2A). They were observed feeding as late as 3 and 4 a.m., respectively. In contrast, IR males and females began feeding between 6 and 7 p.m., but peak activity of the females occurred between 7 and 8 p.m., which was 1 h earlier than the peak feeding of their N counterparts. Peak feeding for both IR and N males occurred at about the same time, but feeding by IR males was more intense between 7 and 9 p.m. than that of the N males at that time.

The feeding pattern of IR males appeared to be more erratic and continued for about 1 h longer than that of N males. The statistical analysis of the feeding data showed a highly significant difference between sexes within strains. Asynchronous feeding patterns between strains were possibly caused by differences in age structure (IR insects were 1 to 2 days old) and in the quality or amount of food supplied to the IR insects before release; however, the difference between sexes within strains suggests a true asynchrony in the time of night that females and males feed.

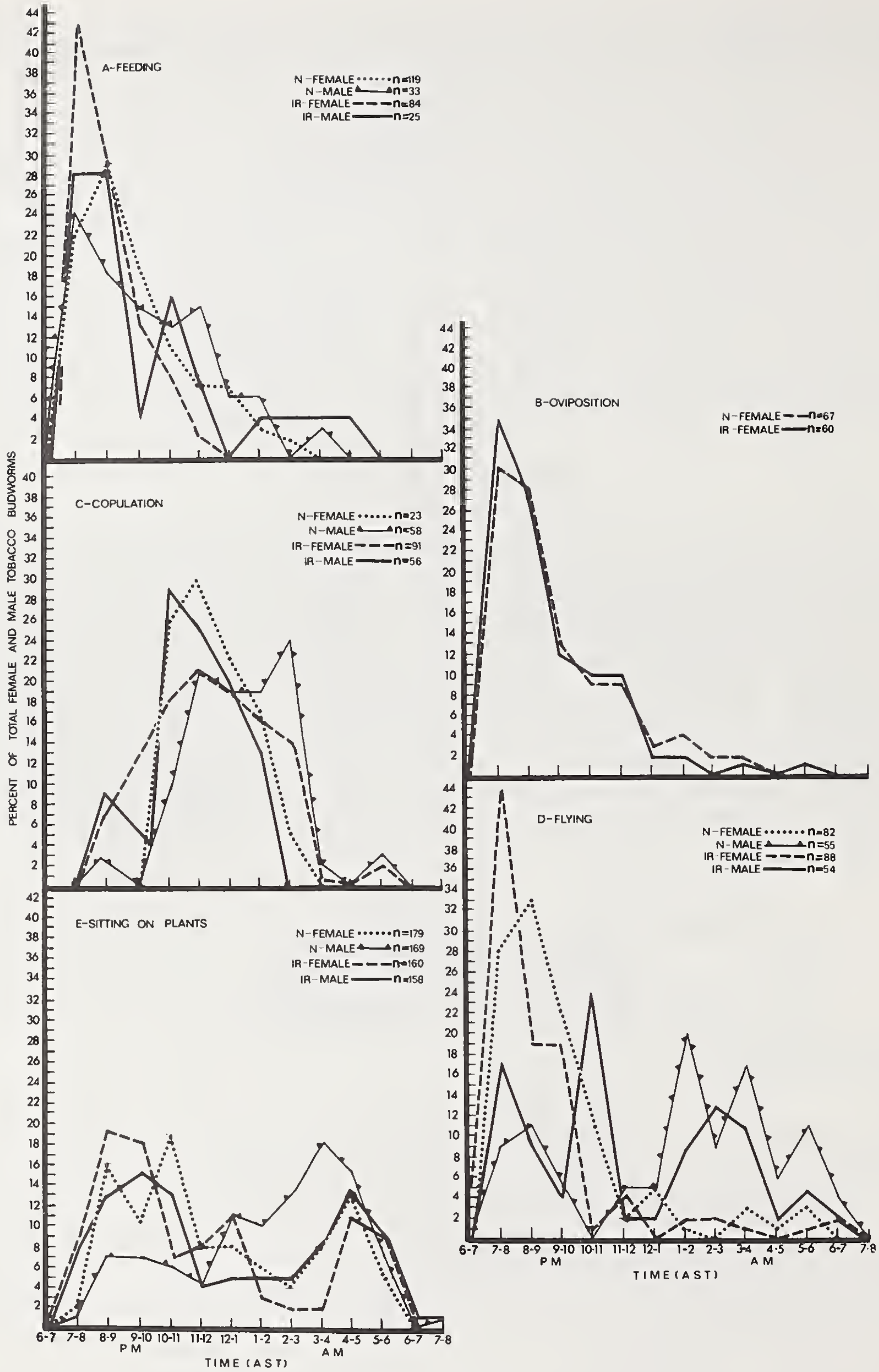


Figure 2.--Diel patterns of nocturnal behavior of native (N) and laboratory-reared irradiated and released (IR), and light-adapted (L) tobacco budworms adults in pigeon pea and *Bastardia* sp. Data reflect one night of observation at Isabela, P.R., and 25 nights of observation on St. Croix, U.S. V.I. All observations were made between April 11 and May 28, 1974. Behavioral traits observed were as follows: A = feeding, B = oviposition, C = copulating, D = flying, and E = sitting on plants.

We observed no significant differences in ovipositional patterns of the two strains. Both N and IR females began ovipositing between 7 and 8 p.m. The activity then continued at relatively high levels until about 9 p.m. (fig. 2B); thereafter, it declined sharply until 1 a.m. after which little oviposition took place.

## Copulation

No mating was observed before 8 p.m., and little occurred before 10:30 p.m. (fig. 2C). Most early matings were between IR adults. Native females began mating between 10 and 11 p.m.; the peak occurred between 11 and 12 p.m., and there was a relatively high level of activity until 2 a.m. After 2 a.m., females mated rarely, and none were observed mating after 3 a.m. In contrast, IR females began mating much earlier (between 8 and 9 p.m.), and numerous IR females were observed mating from 10 p.m. until 3 a.m.; the peak occurred between 11 and 12 p.m. Only three IR females were observed mating after 3 a.m.--one between 3 and 4 a.m. and two between 5 and 6 a.m. The first N male was observed mating (with an IR female) between 8 and 9 p.m. The N males were not observed mating again until about 11 p.m. when activity became intense. Relatively intense mating continued until 3 a.m. Only three N males were observed mating after 3 a.m. IR males began mating between 8 and 9 p.m., and activity peaked between 10 and 11 p.m. No IR males were observed mating after 2 a.m. Matings between N males and IR females were common, and most mating pairs collected after 12 p.m. consisted of IR females and N males. Only one IR male was collected mating with a N female, and the mating occurred at about 2 a.m. Statistical analysis of the data indicated a significant interaction in mating activity of the two strains. There was a highly significant difference in mating activity between IR and N males (IR males mated earlier than N males), but no significant difference in time of mating between IR and N females (though IR females began mating earlier and continued mating longer than N females). Raulston et al. (6) observed asynchrony in the mating patterns of N and IR females of about 2 h. We found the same asynchrony in the present studies, but the N females we collected in copula were too few to be statistically meaningful.

We interpret our results as follows: (1) IR females and males mate earlier than N females and males; (2) males will not respond to females before a given period of the night unless they are very close to a calling female; and (3) IR males do not compete with N males for N females, but IR females compete readily with N females for N males.

## Flying and Sitting

Few females and no males of either strain were observed flying between 6 and 7 p.m. (fig. 2D), but by 8 p.m., flight activity, especially females, had become intense. The flight activity of IR female peaked about 1 h earlier than that of N females and declined earlier. The fact that flight activity of most females occurred between 7 and 10 p.m. indicates that it was probably associated with feeding and oviposition. Some males of both strains were observed flying between 7 and 9 p.m., probably in relation to feeding. Other peak periods for IR males occurred between 10 and 11 p.m. and 2 and 4 a.m. The activity that occurred between 10 and 11 p.m., however, was different from that between 7 and 9 p.m. because the males were flying rapidly above the host plant canopy, probably searching for mates. The flight activity of N males was

similar to that of IR males but the peaks occurred later, which again suggests asynchrony between the two strains.

Statistically, flight activity of females was earlier than that of males ($P < 0.02$). There was no significant interaction.

Very few N adults were observed sitting on plants before 8 p.m. or after 7 a.m. (fig. 2E); most N females were sitting between 8 and 11 p.m., and most N males between 12 p.m. and 5 a.m. The sitting pattern of IR females was similar to that of N females, but IR females were observed sitting earlier than N females ($P < 0.05$); few females were observed on plants between 11 p.m. and 4 a.m., and the frequency of sitting increased between 4 and 5 a.m. The sitting patterns of N and IR males were quite different ($P < 0.001$); relatively large numbers of IR males were observed sitting between 8 and 11 p.m., but few N males were observed sitting during this time.

Analysis of the data shows a highly significant interaction between sex and strain. There was little similarity in the sitting pattern of N males and females, but the numbers of sitting IR females and males was not significantly different except between 2 and 4 a.m. when significantly more IR males than IR females were observed sitting; however, greater numbers of both sexes of the IR strain than of the N strain were present on plants early in the evening, particularly IR males. Perhaps the IR males (only 1 to 2 days old at the time of release) were simply younger and more prone to sit on plants during the early evening hours than their N counterparts.

## Relative Abundance and Sex Ratio at Action Sites

Counts taken on May 13 before the first release at Isabela showed about four times more N females than N males at the action sites (table 1). By May 14, after the first release (IR females), the numbers of N males at the actual release site (Pigeon Pea No. I) had increased about 50-fold, and the numbers at the other action sites had decreased. Throughout the test period, the numbers of N males and females collected at all action sites were about equal (female, 170; male, 166).

After the first release, the numbers of N females at action sites other than at Pigeon Pea No. I declined, probably because we were removing adults as we found them, and they were not being replaced by emergence or immigration. About equal numbers of N females were present in Pigeon Pea No. I and II, but only about one-third as many were present at the other two apparently less attractive action sites.

Most IR adults were collected at the female release site (Pigeon Pea No. I), which was also the site closest to the male release point (table 1). Understandably, more IR females than males (500 vs. 383) were observed at this site since males were released in a field about 110 m away. The sex ratio of IR adults at the other action sites, however, was nearly equal (48 females vs. 49 males). Indeed, a comparison of the relative abundance of the adults collected at action sites other than the female release site shows similar trends in numbers of IR and N adults present. Likely, this relates to the attractancy of the different sites and indicates that the released and N insects dispersed similarly.

TABLE 1.--*Sex ratio and distribution of native (N) and laboratory-reared irradiated and released (IR) tobacco budworm adults at action sites in Isabela, P.R., in 1974, as determined by collecting adults at sites throughout the night*

*[F = female; M = male]*

| Action site | Distance (m) and direction from male release point[3] | Native (N) adults[1] | | | | | | | | | | Released (IR) adults[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 13 | | 14 | | 15 | | 16 | | Total | | 14 | | 15 | | 16 | | Total | |
| | | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M |
| Pigeon Pea No. I | 110 (W.) | 13 | 1 | 14 | 50 | 11 | 55 | 21 | 18 | 59 | 124 | 244 | 175 | 123 | 56 | 85 | 103 | 452 | 334 |
| Pigeon Pea No. II | 261 (S.) | 25 | 10 | 14 | 5 | 12 | 4 | 12 | 5 | 63 | 23 | 6 | 21 | 12 | 7 | 5 | 8 | 23 | 36 |
| Tomato | 420 (SW.) | 16 | 2 | 3 | 8 | 2 | 2 | | | 21 | 12 | 3 | 4 | 5 | 3 | | | 8 | 7 |
| Tobacco | 512 (S-SW.) | | | 13 | 3 | 11 | 2 | 3 | 2 | 27 | 7 | 4 | 3 | 11 | 1 | 2 | 2 | 17 | 6 |
| Totals | | 54 | 13 | 44 | 66 | 36 | 63 | 36 | 25 | 170 | 166 | 257 | 203 | 151 | 67 | 92 | 113 | 500 | 383 |

[1]May 13, 14, 15, and 16.

[2]May 14, 15, and 16; 6,000 of each sex released on May 14, none released on May 15, 2,000 of each sex released on May 16.

[3]All females released at Pigeon Pea No. I; males released about 110 m E.

## Hourly Distribution of Sexes of N and IR adults at Action Sites

A comparison of the number of N adults present each hour at the action sites indicates that more females than males were present or observable during the early evening (fig. 3), and that the hourly distribution of IR and N females was similar. The hourly distribution of the IR males, however, was somewhat different than that of the N males: Relatively more IR males than N males were present in the early evening, but their numbers declined between midnight and 4 a.m. In contrast, the numbers of N males increased from midnight until 3 a.m. Thus, IR males immigrated into the sites and were active earlier in the evening than N males.

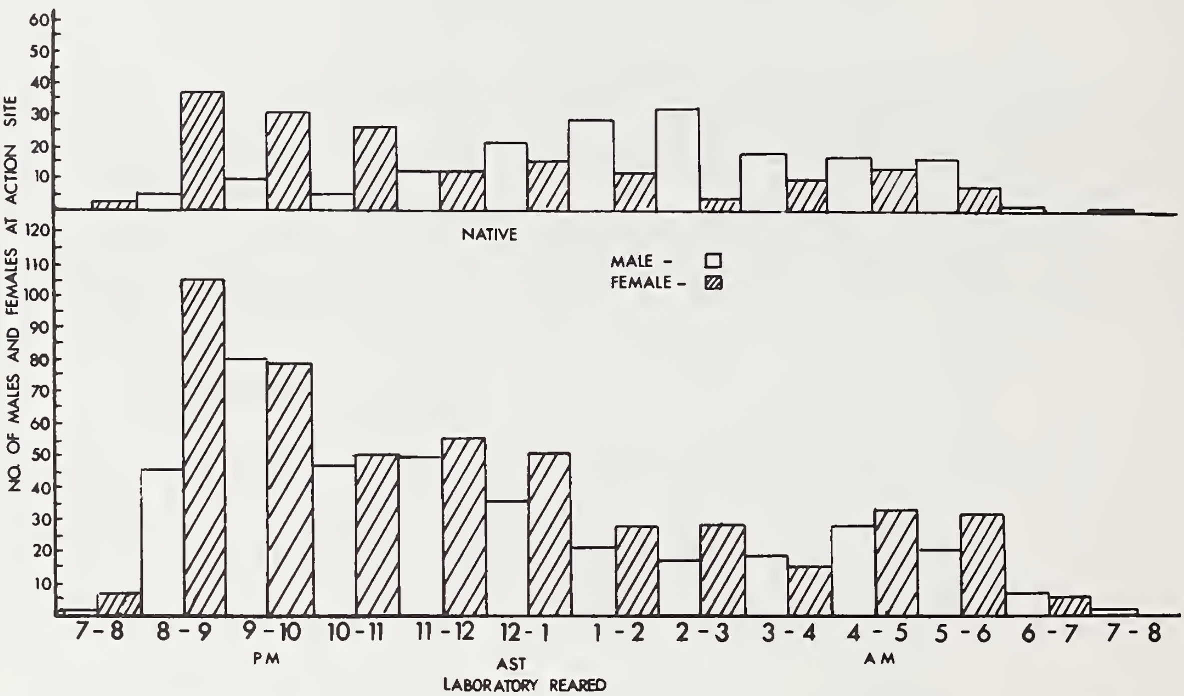


Figure 3.--Numbers of native (N) and laboratory-reared irradiated and released (IR) tobacco budworm adult females and males at action sites during each hour of the night; data based on counts made at Isabela, P.R., on May 13, 14, 15, and 16, 1974.

## Prior Mating Status of N and IR Females

About 85 percent of the N females collected at action sites had previously mated, and 35 percent had mated more than once (table 2). These percentages did not vary significantly over time ($P < 0.5$), suggesting that the N females had mated prior to the test and were not a part of the mating population. The percentage of multiple-mated IR females increased with time ($P < 0.001$); more than 82 percent of them had obtained at least one mate and a few IR females had mated four times although these may have mated before release because of sexing errors. Raulston et al. (*8*), however, established that virgin female tobacco budworms are able to mate during the first night after eclosion and that the

incidence of remating increases with time after the initial mating, though females that receive an adequate amount of eupyrene sperm seldom remate for at least 2 days. Therefore, the IR females that remated (7 percent) in our test were probably ones that mated initially with IR males since we know that such males frequently fail to transfer adequate amounts of eupyrene sperm (*4*). The excess of N males at the IR female release site (table 1) suggests that the mating potential of N males was much greater than that of the N females (85 percent previously mated) (table 2). In fact, only seven N females were collected in copula from May 14 to 16 and only one with an IR male. Meanwhile, 64 N males were collected while copulating with IR females. During the same period, we collected 240 pairs of IR adults in copula.

TABLE 2.--*Mating status of native (N) and laboratory-reared irradiated and released (IR) tobacco budworm females at action sites, Isabela, P.R., 1974* [1] [2]

| Date | Strain | Females | Females [3] Unmated | Females [3] mated | Females [3] Multiple mated | Mating range |
|---|---|---|---|---|---|---|
| | | *Number* | *Percent* | *Percent* | *Percent* | *No. spermatophores per female* |
| May 14 | N | 44 | 10 | 53 | 37 | 0-4 |
| | IR | 257 | 24 | 75 | 1 | 0-2 |
| 15 | N | 36 | 9 | 57 | 34 | 0-6 |
| | IR | 151 | 16 | 74 | 10 | 0-3 |
| 16 | N | 36 | 24 | 43 | 33 | 0-4 |
| | IR | 92 | 4 | 78 | 18 | 0-4 |
| Total | N | 116 | 14 | 51 | 35 | 0-6 |
| | IR | 500 | 18 | 75 | 7 | 0-4 |

[1]Six thousand IR adults of each sex released on May 14; 2,000 of each sex released on May 16.

[2]Based on single female collections, and includes all females collected as copulating pairs.

[3]Both IR sexes dark-adapted before release on May 14, males light-adapted and females dark-adapted on May 16.

## Effects of Dark-Adaptation (D) of IR Adults on Mating Interaction With N Population

Two-day-old IR females (dark-adapted for 4 h prior to release near sundown at Pigeon Pea No. I on May 14) were calling intensely at the time of release (7 p.m.). When D-IR males were released at 7:15 p.m., they flew at least 110 m crosswind to Pigeon Pea No. I, and copulating pairs of D-IR adults were

observed shortly thereafter (table 3). A majority of the D x D matings occurred about 2 h earlier than matings between other possible combinations ($P < 0.01$). The L x L matings occurred earlier than N x N matings though too few N x N matings were observed to allow any statistical inferences, but Raulston et al. (*6*) previously reported a significant asynchrony in mating between the two strains of tobacco budworms.

TABLE 3.--*Pattern of mating response of native (N) vs. released laboratory-reared, irradiated (IR), light- (L), and dark-adapted (D) tobacco budworm adults at action sites, Isabela, P.R., 1974*[1]

| Time (AST) | Numbers of various combinations (female x male) copulating at times indicated[2 3] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | D x D | L x L | N x N | D x L[4] | L x N | N x L | D x N | N x D |
| 7-8 p.m. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-9 | 25 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| 9-10 | 68 | 1 | 0 | 2 | 0 | 0 | 1 | 1 |
| 10-11 | 22 | 10 | 0 | 7 | 0 | 0 | 0 | 0 |
| 11-12 | 23 | 8 | 0 | 16 | 5 | 0 | 1 | 1 |
| 12-1 a.m. | 6 | 9 | 2 | 16 | 4 | 0 | 7 | 0 |
| 1-2 | 6 | 2 | 2 | 16 | 6 | 1 | 9 | 0 |
| 2-3 | 0 | 4 | 1 | 0 | 7 | 0 | 14 | 0 |
| 3-4 | 0 | 0 | 1 | 2 | 3 | 0 | 1 | 0 |
| 4-5 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 5-6 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 6-7 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | 156 | 35 | 6 | 49 | 29 | 1 | 35 | 2 |

[1]Six thousand dark-adapted males and females released on May 14, 2,000 light-adapted males and 2,000 dark-adapted females released on May 16; collections made on May 13, 14, 15, and 16.

[2]L x D combination not tested.

[3]Females listed first.

[4]Likely includes some light-adapted females.

The time that L males responded to L or D females (table 3) did not differ significantly, indicating that male tobacco budworms do not respond to females until a given period regardless of calling activity of females. The response times of N males showed similar trends. Indeed, few N or L males responded to either L or D females before the normal periods of intense mating activity though D males did respond to the early calling D females. Thus, males generally do not respond to females until a given period of the night unless they are extremely close to a calling female. The fast flight syndrome associated with male activity (fig. 2D) tends to support this conclusion since this type of flight begins prior to calling by females and is apparently related to male searching behavior.

D-IR females placed on mating tables near sundown called significantly earlier than L females (table 4). N males responded to the D females about 1 h earlier than they responded to L females; however, none responded before 11 p.m., which is the normal time of response of N males to N females.

TABLE 4.--*Response of native males to calling of laboratory-reared irradiated clipped-wing, dark-, and light-adapted virgin tobacco budworm females on mating tables as determined by time of copulation*

| Time (AST) | Percent females calling and number mating at time indicated[1] | | | |
|---|---|---|---|---|
| | Dark adapted | | Light adapted | |
| | Calling | Mating | Calling | Mating |
| 8 p.m. | 10 | 0 | 1 | 0 |
| 9 | 17 | 0 | 8 | 0 |
| 10 | 15 | 0 | 9 | 0 |
| 11 | 28 | 4 | 8 | 0 |
| 12 | 53 | 8 | 30 | 5 |
| 1 a.m. | 20 | 2 | 43 | 7 |
| 2 | 5 | 0 | 29 | 7 |
| 3 | 2 | 0 | 10 | 0 |
| 4 | 0 | 0 | 12 | 0 |

[1] One hundred fifty clipped-wing females of each type (3 tables containing 50 females per table).

From our observations, released males were not able to obtain N mates though they mated readily with both L- and D-IR females. As previously noted, the N female population was heavily mated (table 2) and therefore relatively old; as a consequence, we observed only a few N x N matings. This lack of N females susceptible to mating and the asynchrony between the N and IR populations may account to some extent for the failure of IR male entry into the N female mating population. Even so, Raulston et al. (*6*) have clearly demonstrated that even unirradiated, laboratory-reared tobacco budworm males cannot enter into an N female population. Unfortunately, the prior mating status of the population they examined was unknown so the cause of the failure was not shown. Further, unpublished data collected by the senior author indicates that N females of several lepidopteran species reject IR males because of an odor associated with wheat germ base diets.

## Response of Males to Females on Mating Tables

At Pigeon Pea No. I, only one N male responded to IR females before 11 p.m., and mating peaked between 2 and 3 a.m. (table 5). IR males were mating readily with IR females at this action site by 11 p.m., and their mating peaked between 10 p.m. and 1 a.m. On mating tables at action sites, no males responded to females before 9 p.m., but IR males responded earlier to females

TABLE 5.--*Effect of location of mating tables baited with laboratory-reared and irradiated (IR), clipped-wing, virgin tobacco budworm light-adapted females on time and degree of mating of native (N) males and released IR males*

| Time (AST) | Males responding to light-adapted females at indicated distance and direction from male release point[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Free females at Pigeon Pea No. I[2] | | Tomato and tobacco (402-512 m SW.) | | Pigeon Pea No. II (261 m S-SW.) | | Pigeon Pea No. III (648 m SW.) | | Plowed field (484 m N-NW.) | | Pasture (784 m NE.) | |
| | N | IR | N | IR | N | IR | N | IR | N | IR | N | IR |
| | *Percent* | | | | | | | | | | | |
| 8-9 p.m. | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0-10 | 0 | 3 | 0 | 11 | 33 | 17 | 0 | 12 | 0 | 0 | 0 | 0 |
| 10-11 | 0 | 26 | 7 | 21 | 0 | 23 | 6 | 14 | 0 | 15 | 0 | 0 |
| 11-12 | 16 | 24 | 23 | 23 | 0 | 18 | 14 | 5 | 26 | 44 | 0 | 0 |
| 12-1 a.m. | 17 | 26 | 27 | 24 | 67 | 34 | 37 | 54 | 30 | 15 | 72 | 50 |
| 1-2 | 20 | 6 | 13 | 15 | 0 | 0 | 17 | 0 | 33 | 25 | 28 | 50 |
| 2-3 | 30 | 12 | 30 | 6 | 0 | 8 | 17 | 15 | 11 | 2 | 0 | 0 |
| 3-4 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-5 | 0 | 0 | - | - | - | - | - | - | - | - | - | - |
| 5-6 | 7 | 0 | - | - | - | - | - | - | - | - | - | - |
| 6-7 | 0 | 0 | - | - | - | - | - | - | - | - | - | - |
| No. males[3] | 30 | 34 | 30 | 84 | 3 | 82 | 35 | 44 | 43 | 48 | 18 | 2 |
| Total females | 36 | 151 | 300 | | 150 | | 150 | | 150 | | 100 | |
| Percent females mating | [4]0 | 43 | 38 | | 57 | | 53 | | 61 | | 20 | |

[1]Fifty clipped-wing females per table per night (3 nights per table except 2 nights per table in pasture); nights of May 14, 15, and 16, except May 14 and 15 for table in pasture.

[2]Numbers of females based on head-counts taken at Pigeon Pea No. I on releases made on previous night. Night of May 15 on which no males or females were released to insure that both sexes of each strain were light adapted. Site of female releases located 110 m W. of male release site.

[3]N and IR males mated with IR females only.

[4]No N females were observed mating with N or IR males on night of May 15.

there than they responded to IR females on host plants (Pigeon Pea No. I). On mating tables away from action sites, males responded to females about 2 to 4 h later than to females on tables at action sites, but the IR males responded 1 h earlier than N males to the clipped-wing females on tables away from action sites (plowed field) (table 5). Indeed, both types of males responded to clipped-wing females on mating tables located from 484 to 784 m from the IR male release site, but the response occurred much later than the response to released females at action sites or to clipped-wing females on tables at action sites. A statistical analysis of the data shows a highly significant difference in the time when both male types responded to IR females at action sites, to clipped-wing IR females located on tables at action sites, and to clipped-wing IR females located on tables away from action sites.

Both N and IR males obviously search out action sites before they search out areas that do not contain attractive host plants. Males appear to respond earlier to concentrations of females on tables at action sites than to more dispersed released females at action sites. This difference is likely related to differences in the plume of pheromone around a table (50 females) compared with that from single females on plants.

## SUMMARY

Observations of the nocturnal behavior of N and IR females and males showed asynchrony in diel patterns of feeding, copulation, flying, and sitting between strains and between sexes within strains. This asynchrony appears to affect mating within strains and mating competitiveness between the two strains. IR males mated earlier than N males and were unable to obtain N female mates, but N males mated readily with IR females. The degree of mating interaction between the two strains was influenced greatly by the prior mating status of the N females (age structure of N population), and a heavily mated (old) N population, thus resulting in a disproportionate N male mating potential (greater than that of the N female even though the sex ratios are about equal).

Females of both strains tended to stay at action sites throughout the night; males dispersed readily throughout the ecosystem regardless of the presence or absence of attractive host plants. The dispersal, however, was closely related to the search for females. This behavior appears to be independent of the presence of pheromone. Male movement into areas with low concentrations of host plants usually occurred late at night after the males had searched out action sites.

Dark-adaptation of females and males resulted in earlier calling and mating, but released males exposed to normal photoperiods and N males both responded to D females at about the same time they responded to L and N females. They therefore demonstrated again that the time of male response to females depends on male searching behavior and is generally independent of the presence or absence of pheromone except when the females are at very close range. Nevertheless, the initiation of male search and of pheromone secretion by females of a given strain is closely synchronized.

Concentrations of IR females at action sites tended to produce concentrations of N males, and concentrations of clipped-wing females on matching table (50 per table) at action sites caused an earlier male response than did free IR females at action sites. Apparently, the concentrations of IR females on tables produced a larger plume of pheromone than free IR females dispersed over plants at action sites, and thus the searching males came to the table first.

Mating tables can be used to monitor male dispersal and male response to females, but the time and degree of response is greatly influenced by availability of attractive host plants at the site where tables are located and the relative distance from action sites. Therefore, data from mating tables only give information on male dispersal and time of mating response. They tell very little about the mating interaction between IR and N adults that occurs following release.

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